NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 457

TOXICOLOGY AND CARCINOGENESIS STUDIES OF SALICYLAZOSULFAPYRIDINE

(CAS NO. 599-79-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

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P.O. Box 12233
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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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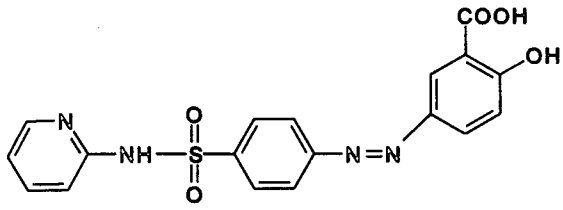
ABSTRACT



SALICYLAZOSULFAPYRIDINE

CAS No. 599-79-1

Chemical Formula: $C_{18}H_{14}N_4O_5S$ Molecular Weight: 398.39

Synonyms: 2-Hydroxy-5-[[4-[2-(pyridinylamino)sulfonyl]phenyl]azo]benzoic acid; 4-(pyridyl-2-amidosulfonyl)-3′-carboxy-4′-hydroxyazobenzene; 5-[4-(2-pyridylsulfamoyl)phenylazo]-2-hydroxybenzoic acid; 5-[*p*-(2-pyridylsulfamoyl)phenylazo]salicylic acid; salazosulfapyridine; sulfasalazine; sulphasalazine

Trade names: Azopyrin, Azulfidine, Benzosulfa, Colo-Pleon, Reupirin, Salazopyrin

Salicylazosulfapyridine is widely used for the treatment of ulcerative colitis and Crohn's disease. It has been beneficial in the treatment of psoriasis and rheumatoid arthritis, and it has been used in veterinary medicine for the treatment of granulomatous colitis. Salicylazosulfapyridine was nominated for toxicity and carcinogenicity testing by the National Cancer Institute on the basis of its widespread use in humans and because it is a representative chemical from a class of aryl sulfonamides. Salicylazosulfapyridine is a suspect carcinogen because reductive cleavage of the azo linkage yields a *p*-amino aryl sulfonamide (sulfapyridine), and a related *p*-amino aryl sulfonamide (sulfamethoxazole) has been shown to produce thyroid neoplasms in rats. Toxicology and carcinogenicity studies were conducted in F344/N rats and B6C3F$_1$ mice. Rats and mice were administered salicylazosulfapyridine (96% to 98% pure) in corn oil by gavage for 16 days, 13 weeks, or 2 years. The gavage route of administration was selected for these studies because it approximates the typical route of human exposure to the chemical. Genetic toxicology studies were conducted *in vitro* in *Salmonella typhimurium* and cultured Chinese hamster ovary cells and *in vivo* in rat and mouse bone marrow and mouse peripheral blood cells.

16-Day Study in Rats

Groups of five male and five female rats were administered 0, 675, 1,350, or 2,700 mg salicylazosulfapyridine/kg body weight in corn oil by gavage for 16 days excluding weekends. All rats survived to the end of the study. With the exception of the 675 mg/kg male group, the final mean body weights of all dosed groups of males and females were significantly lower than those of controls. Mean body weight gains of all dosed groups were less than those of controls. Clinical findings included ruffled fur and distended abdomens in male and female rats receiving 2,700 mg/kg.

Hypothyroidism, evidenced by decreased serum triiodothyronine and thyroxine concentrations and increased thyroid-stimulating hormone concentrations, occurred in 2,700 mg/kg male and female rats. The absolute and relative thymus weights of male rats receiving 1,350 or 2,700 mg/kg and female rats

receiving 2,700 mg/kg were significantly lower than those of controls.

At necropsy, all dosed rats had enlarged cecae/large intestines. Male rats receiving 1,350 mg/kg and male and female rats receiving 2,700 mg/kg had red, enlarged thyroid glands. Chemical-related microscopic lesions were present in the forestomach, thymus, thyroid gland, and pituitary gland. Minimal to mild hyperplasia of the forestomach mucosa was present in the 1,350 and 2,700 mg/kg male and female groups. Lymphoid depletion was observed in the thymus of three male and three female rats in the 2,700 mg/kg groups. Male and female rats receiving 1,350 and 2,700 mg/kg had thyroid gland follicular cell hyperplasia and an increase in thyroid-stimulating hormone producing cells in the pars distalis of the pituitary gland.

16-Day Study in Mice

Groups of five male and five female mice were administered 0, 675, 1,350, or 2,700 mg salicylazosulfapyridine/kg body weight in corn oil by gavage for 16 days excluding weekends. There were no chemical-related deaths, and final mean body weights of dosed mice were similar to those of controls. No chemical-related clinical findings were noted for male or female mice. There were no differences in triiodothyronine, thyroxine, or thyroid-stimulating hormone concentrations between dosed and control mice. There were no biologically significant differences in absolute or relative organ weights between dosed and control male and female mice. At necropsy, male mice receiving 2,700 mg/kg had enlarged cecae/large intestines. There were no biologically significant histopathologic lesions attributed to salicylazosulfapyridine administration.

13-Week Study in Rats

Groups of 10 male and 10 female rats were administered 0, 84, 168.8, or 337.5 mg salicylazosulfapyridine/kg body weight in corn oil by gavage for 13 weeks. All rats survived to the end of the study. The final mean body weights of dosed male rats were similar to those of controls; the final mean body weights and body weight gains of dosed females were significantly lower than those of controls. No chemical-related clinical findings were noted in dosed male or female rats during the 13-week study. No significant differences in hematology or urinalysis parameters between control and dosed rats were observed. The absolute and relative right kidney weights of 337.5 mg/kg females were significantly greater than those of controls.

At necropsy, some 337.5 mg/kg male rats had red, enlarged thyroid glands. Histopathologic changes were noted primarily in the thyroid gland and pituitary gland of males and females in the 337.5 mg/kg groups. The thyroid gland lesions observed were similar to those present in the 16-day study. Nine male rats receiving 168.8 mg/kg and ten male and seven female rats receiving 337.5 mg/kg had minimal but consistent changes in thyroid gland follicular cells. In the pituitary gland of 337.5 mg/kg males and females, the thyroid-stimulating hormone producing cells were enlarged and contained pale-staining cytoplasm and prominent Golgi complexes. Decreased serum triiodothyronine and thyroxine concentrations and increased thyroid-stimulating hormone concentration, similar to differences observed in the 16-day study, occurred in 337.5 mg/kg male rats; thyroid hormone concentrations were not affected in female rats.

Sperm motility of all dosed groups of males was significantly lower than that of controls. Vaginal cytology parameters of dosed groups of females were similar to those of controls.

13-Week Study in Mice

Groups of 10 male and 10 female mice were administered 0, 675, 1,350, or 2,700 mg salicylazosulfapyridine/kg body weight in corn oil by gavage for 13 weeks. All mice survived to the end of the study. The final mean body weights of dosed male and female mice were similar to those of controls. The mean body weight gains of 1,350 and 2,700 mg/kg male mice were less than that of controls. No chemical-related clinical findings were noted in dosed male or female mice during the 13-week study.

There was minimal evidence of a responsive anemia in mice in the 13-week study. The anemia was probably related to a methemoglobinemia. There were minimal decreases in thyroxine concentration in all dosed groups of male and female mice in the 13-week

study. There were, however, no differences in triiodothyronine and thyroid-stimulating hormone concentrations between dosed and control animals.

Absolute and relative liver weights of all groups of dosed male and female mice were significantly greater than those of controls. There were no chemical-related gross lesions. Microscopic evaluation of the liver revealed centrilobular hypertrophy in five 1,350 mg/kg and all 2,700 mg/kg male mice.

The right cauda weight of the 1,350 mg/kg group and the right epididymis weights of all dose groups were significantly lower than those of controls. There was no evidence of chemical-related alteration in the vaginal cytology parameters of female mice.

2-Year Study in Rats

Groups of 60 male and 60 female rats were administered 84, 168, or 337.5 mg salicylazosulfapyridine/kg body weight in corn oil by gavage for up to 105 weeks. Groups of 70 male and 60 female rats were administered the corn oil vehicle by gavage for up to 105 weeks. A stop-exposure group of 70 male rats was administered 337.5 mg/kg salicylazosulfapyridine in corn oil by gavage for 6 months, after which animals received the corn oil vehicle by gavage for the remainder of the 2-year study. Ten animals from the vehicle control male group and 10 animals from the 337.5 mg/kg stop-exposure group were evaluated at 6 months; 10 animals from each core-study group were evaluated at 15 months.

Survival, Body Weights, and Clinical Chemistry

Survival of 337.5 mg/kg male core-study rats was significantly lower than that of controls; survival of 84 and 168 mg/kg core-study males, all groups of dosed females, and the stop-exposure male group was similar to controls. Mean body weights of core-study males and stop-exposure males were similar to controls throughout the study. From week 45 to the end of the study, females in the 337.5 mg/kg group had mean body weights that were lower than those of controls. The serum thyroxine concentration in 337.5 mg/kg core-study males at study termination was minimally lower than that of controls; the serum thyroid-stimulating hormone, triiodothyronine, and reverse triiodothyronine concentrations of dosed males and females were similar to those of controls.

Pathology Findings

Administration of salicylazosulfapyridine for 2 years was associated with transitional epithelial papilloma in the urinary bladder of male rats and may have been associated with transitional epithelial papilloma of the kidney and of the urinary bladder of female rats. Nonneoplastic effects in the urinary bladder and kidney of male and female rats and in the spleen of male rats were also observed. Dosed male and female rats had increased incidences of grossly and microscopically observed urinary bladder concretions (diagnosed grossly as calculi at necropsy); male and female rats that developed transitional epithelial papillomas of the urinary bladder had grossly observed concretions (calculi) in the urinary bladder at necropsy. The microscopic neoplastic and nonneoplastic urinary bladder and kidney effects observed in dosed male rats during the 2-year continuous study did not occur in dosed rats during the 2-year stop-exposure study, nor were there gross observations of concretions (calculi) at necropsy. The incidences of mononuclear cell leukemia in male and female rats were decreased. The thyroid gland hyperplasia seen in the 13-week study was not observed in the 2-year study, and there was no evidence of chemical-related thyroid gland follicular cell adenomas or carcinomas.

2-Year Study in Mice

Groups of 60 male and 60 female mice were administered 0, 675, 1,350, or 2,700 mg salicylazosulfapyridine/kg body weight in corn oil by gavage for up to 104 weeks. Ten animals from each group were evaluated at 15 months.

Survival, Body Weights, and Clinical Chemistry

Survival of all the dosed groups of male and female mice was similar to that of controls. Mean body weights of 675 and 1,350 mg/kg male and female mice were similar to controls throughout the study. From week 12 to the end of the study, 2,700 mg/kg male mice had mean body weights that were lower than those of controls. From week 14 to the end of the study, the 2,700 mg/kg female mice had mean body weights that were lower than those of controls.

There were no chemical-related differences in triiodothyronine, reverse triiodothyronine, thyroxine, or thyroid-stimulating hormone concentrations between dosed and control mice at the 15-month evaluation.

Pathology Findings

Exposure of mice to salicylazosulfapyridine in corn oil by gavage for 2 years was associated with increased incidences of hepatocellular neoplasms in males and females. Nonneoplastic effects in the liver and spleen were also observed in male and female mice. The incidences of forestomach squamous cell papilloma in females and forestomach hyperplasia in males and females were decreased.

GENETIC TOXICOLOGY

Salicylazosulfapyridine was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535, and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. These *in vitro* assays were performed with and without S9 metabolic activation enzymes.

Results from *in vivo* mouse bone marrow chromosomal aberration tests were uniformly negative, while results of micronucleus assays performed on male or female mice exposed to salicylazosulfapyridine for periods ranging from 3 days to 13 weeks were positive. Micronucleus tests in male mice for shorter exposure times (1 to 2 days) yielded negative or very weakly positive results. A three-treatment (72-hour exposure time) micronucleus test performed in male rats yielded equivocal results. Overall, results of these *in vivo* assays indicate that salicylazosulfapyridine is capable of inducing chromosomal damage, possibly in the form of aneuploidy, in mouse bone marrow cells after multiple administrations.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity** of salicylazosulfapyridine in male and female F344/N rats based on increased incidences of neoplasms in the urinary tract. There was an increased incidence of transitional epithelial papilloma of the urinary bladder in males and a low incidence of rare transitional epithelial papillomas of the kidney and of the urinary bladder in females. There was *clear evidence of carcinogenic activity* of salicylazosulfapyridine in male and female B6C3F_1 mice based on increased incidences of hepatocellular neoplasms.

Increased incidences of nonneoplastic lesions of the urinary bladder and kidney in male and female rats and of the spleen in male rats were observed. Increased incidences of nonneoplastic lesions of the liver and spleen in male and female mice were observed.

Decreased incidences of mononuclear cell leukemia in male and female rats were related to salicylazosulfapyridine administration. Decreased incidences of forestomach squamous cell papilloma in female mice and forestomach hyperplasia in male and female mice were related to salicylazosulfapyridine administration.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Salicylazosulfapyridine

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 84, 168, or 337.5 mg/kg, or 337.5 mg/kg stop-exposure	0, 84, 168, or 337.5 mg/kg	0, 675, 1,350, or 2,700 mg/kg	0, 675, 1,350, or 2,700 mg/kg
Body weights	Dosed groups similar to controls	337.5 mg/kg group lower than controls	2,700 mg/kg group lower than controls	2,700 mg/kg group lower than controls
Survival rates	35/50, 33/50, 31/50, 23/50, 30/50	35/50, 34/50, 31/50, 26/50	40/50, 41/50, 41/50, 46/50	41/50, 41/50, 37/50, 38/50
Nonneoplastic effects	Urinary bladder: mucosal hyperplasia (0/50, 0/49, 14/50, 41/50, 1/47); concretion (0/50, 0/49, 1/50, 10/50, 0/47); dilatation (0/50, 1/49, 2/50, 7/50, 0/47) Kidney: concretion (0/50, 1/50, 13/50, 33/50, 0/50); transitional epithelial hyperplasia (10/50, 10/50, 20/50, 43/50, 4/50); hydronephrosis (0/50, 1/50, 1/50, 28/50, 0/50); mineralization (3/50, 10/50, 11/50, 13/50, 3/50); renal tubule dilatation (0/50, 1/50, 1/50, 11/50, 3/50) Spleen: hemosiderin pigment (14/50, 15/50, 15/50, 30/50, 13/50); hematopoietic cell proliferation (14/50, 15/50, 17/50, 23/50, 14/50)	Urinary bladder: mucosal hyperplasia (2/49, 0/50, 4/50, 12/50) Kidney: concretion (0/50, 9/50, 34/50, 37/50); transitional epithelial hyperplasia (3/50, 7/50, 23/50, 43/50); hydronephrosis (0/50, 0/50, 3/50, 10/50); nephropathy (34/50, 36/50, 41/50, 44/50)	Liver: eosinophilic foci (6/50, 19/50, 20/50, 22/50) Spleen: hematopoietic cell proliferation (11/50, 16/50, 20/49, 13/50); hemosiderin pigment (2/50, 25/50, 32/49, 47/50)	Liver: eosinophilic foci (5/50, 17/50, 15/50, 19/49) Spleen: hematopoietic cell proliferation (7/50, 21/50, 19/50, 23/49); hemosiderin pigment (14/50, 37/50, 39/50, 46/49)
Neoplastic effects	Urinary bladder: transitional epithelial papilloma (0/50, 0/49, 2/50, 6/50, 0/47)	Kidney: transitional epithelial papilloma (0/50, 0/50, 0/50, 2/50) Urinary bladder: transitional epithelial papilloma (0/49, 0/50, 2/50, 0/50)	Liver: hepatocellular adenoma (13/50, 32/50, 28/50, 42/50); hepatocellular adenoma or carcinoma (24/50, 38/50, 38/50, 44/50)	Liver: hepatocellular adenoma (12/50, 28/50, 25/50, 28/49); hepatocellular carcinoma (2/50, 10/50, 10/50, 9/49); hepatocellular adenoma or carcinoma (14/50, 32/50, 28/50, 29/49)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Salicylazosulfapyridine (continued)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Decreased incidences	Multiple organs: mononuclear cell leukemia (13/50, 18/50, 12/50, 3/50, 10/50)	Multiple organs: mononuclear cell leukemia (14/50, 9/50, 8/50, 3/50)	Forestomach: hyperplasia (18/50, 11/50, 11/49, 10/50)	Forestomach: squamous cell papilloma (5/50, 1/50, 1/50, 0/50); hyperplasia (12/50, 6/49, 4/50, 4/49)
Level of evidence of carcinogenic activity	Some evidence	Some evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative with and without S9 in strains TA97, TA98, TA100, and TA1535			
Sister chromatid exchanges				
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Chromosomal aberrations				
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Mouse bone marrow *in vivo*:	Negative in standard and nonstandard protocols			
Micronucleated erythrocytes				
Mouse bone marrow *in vivo*:	Positive in two standard protocol assays and in kinetochore assay			
Rat bone marrow *in vivo*:	Equivocal			
Mouse peripheral blood *in vivo*:	Positive			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on salicylazosulfapyridine on 20 June 1995 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 20 June 1995, the Technical Report on the toxicology and carcinogenesis studies of salicylazosulfapyridine received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. F.W. Kari, NIEHS, introduced the toxicology and carcinogenesis studies of salicylazosulfapyridine by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. Dr. Kari reported that the gross and morphological evidence of thyroid gland hyperplasia in the 16-day and 13-week studies in rats was confirmed by clinical chemistry and indicated a derangement of the pituitary-thyroid axis. As a result, a stop-exposure study was designed in male rats, which were exposed to the highest dose of salicylazosulfapyridine for six months and given the corn oil vehicle for the remainder of the 2-year term. The expected thyroid gland lesions were not seen in any of the animals carried to 2 years. The proposed conclusions for the 2-year studies were *some evidence of carcinogenic activity* in male and female F344/N rats and *clear evidence of carcinogenic activity* in male and female B6C3F$_1$ mice.

Dr. Ward, a principal reviewer, agreed with the proposed conclusions. He thought that male rats might have tolerated a higher dose. Dr. Kari agreed. Dr. Ward said the stop-exposure studies had limited significance in that neither preneoplastic nor neoplastic lesions were shown to have occurred at 26 weeks. Dr. M.R. Elwell, NIEHS, commented that based on the hyperplasia and thyroid gland effects at 13 weeks, it was reasonable to expect that they would be present at 26 weeks.

Dr. Goldsworthy, the second principal reviewer, agreed with the proposed conclusions but had concerns with the dose selection for mice. Given that liver weight changes and centrilobular hypertrophy were observed in all doses in the 13-week studies, he questioned why a dose at which changes had not been seen was not chosen for the 2-year studies. Dr. Elwell said that at the low dose in the 13-week studies, hypertrophy could not be detected morphologically in female mice, and there was only a 10% to 15% increase in liver weight in males. Dr. Goldsworthy commented that the same rationale given for stop-exposure studies in rats could have been applied to the mouse and mouse liver neoplasms. Dr. Kari reported that at the time the studies were designed, the thyroid gland hyperplasia appeared to be the predominant effect from salicylazosulfapyridine, and further, there was great interest in the role of goitrogenic compounds such as the aryl sulfonamides in endocrine disruption and thyroid gland neoplasia.

Dr. Russo, the third principal reviewer, agreed with the proposed conclusions. She noted that review of the literature indicated the probability of fetal damage in humans from salicylazosulfapyridine, as well as transplacental transport and secretion in milk. Lack of data on absorption and disposition of salicylazosulfapyridine in pregnant experimental animals suggests that future studies of transplacental effects would be desirable.

A discussion ensued about the renal neoplasms and their association with bladder calculi (concretions) in rats. Dr. J.R. Bucher, NIEHS, noted that this association was strongly supported by the fact that every animal with a bladder or kidney papilloma also had grossly observable calculi. Dr. Reddy inquired as to the nature of the concretions. Dr. Kari responded that they were spiculated in nature, but were not chemically analyzed. The calculi were presumed to be precipitated drug and/or metabolites. Dr. Russo asked how these findings could be extrapolated to humans when the doses in animals were so much greater. Dr. Kari said the experiments in rodents were designed to maximize the probability for identifying toxicity and carcinogenicity. He pointed out that blood levels in the rodents used in these and other studies were discussed in this Technical Report and allow comparison with blood levels reported after maintenance doses in humans. At least for some of the doses, comparable blood levels were observed between the species.

Dr. A. Imondi, Director of Toxicology and Safety Assessment, Pharmacia, Inc., stated that after many

years of extensive clinical use as a therapeutic agent in inflammatory bowel disease and rheumatoid arthritis, there is no evidence that salicylazosulfapyridine causes neoplasia in humans. With regard to positive micronucleus tests observed in mice by the NTP, he cited unpublished studies that indicate folic acid deficiency is involved and increased incidences of micronuclei could be reduced or reversed by folic acid supplements to drug treatment. Dr. Imondi described other unpublished studies showing that salicylazosulfapyridine did not produce increases in DNA adduct formation in target tissues. He concluded that clinical experience indicated no relevance of the NTP neoplasm findings to humans.

Dr. Ward moved that the Technical Report on salicylazosulfapyridine be accepted with the revisions submitted by the panelists and with the conclusions as written. Dr. Russo seconded the motion, which was accepted unanimously with ten votes.